Journal of Medical Virology 50:168–175 (1996)

Detection and Clinical Features of Hepatitis C Virus Type 6 Infections in Blood Donors From Hong Kong

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The genotype distribution of hepatitis C virus (HCV) was investigated in 212 viraemic blood donors from Hong Kong. A subset of the samples was investigated using three different genotyping assays to establish the accuracy of each in this population. These assays were restriction fragment length polymorphism (RFLP) of amplified 5′ noncoding region (5′NCR) sequences, RFLP of the core region, and a serotyping assay using peptides from two antigenic regions of NS4.

Genotypes detected in Hong Kong blood donors were 1a (6.2%), 1b (58.8%), 2a (1.4%), 2b (1.4%), 3a (1.9%), and 6a (27.0%). All genotyping assays produced concordant results. No evidence was obtained for the presence of type 6 group variants recently identified in Southeast Asia, other than type 6a. A serotyping assay based upon the detection of type-specific antibody to epitopes in NS4 produced similar results to the genotyping assays (98% concordance), but a reduced sensitivity (75%) compared with genotyping methods. Sequence variation in NS4 was not the cause of the reduced rate of detection of type 6 antibody in this population.

Eighty-four percent donors infected with type 6a were male, compared to 75% donors infected with type 1b. The median alanine transaminase (ALT) level in type 6 infected donors was lower than in type 1b, (43.8 and 51.1 U/l, respectively) although these values were not statistically significant ($P = 0.094$). There was no significant difference between the ages of donors infected with types 1b and 6a. Risk factors for HCV infection in the blood donors included blood transfusion, intravenous drug abuse, and tattooing. A significantly greater number of donors infected with HCV-6a reported a history of drug abuse (66%) than donors infected with HCV-1b (7%).

KEY WORDS: HCV, genotypes, serotypes, blood donor, Southeast Asia

INTRODUCTION

Hepatitis C virus (HCV) is the primary aetiological agent of posttransfusion non-A, non-B hepatitis worldwide. The genome comprises single-stranded, positive sense RNA of approximately 9,400 nucleotide bases in length. As with many other RNA viruses, HCV displays extensive sequence variation. At least six major genotypes have been described to date, each of which can be further divided into subtypes according to their degree of sequence similarity to each other, and by their branching order using phylogenetic analysis [Simmonds et al., 1993a, 1994; Bukh et al., 1993]. For example, there is approximately 70% overall sequence identity between the major genotypes and 80% between subtypes. Infections in the United States and western Europe are generally caused by HCV genotypes 1, 2, and 3, although these are also distributed worldwide. Type 4 is found in Central Africa and in the Middle East, while type 5 is common only in South Africa. Of the six major genotypes, type 6a shows one of the most confined geographical locations, having been found only in Hong Kong, Macau, and Vietnam [Mellor et al., 1996; Tokita et al., 1994; Simmonds et al., 1993a], or in emigrants from these countries [Murphy et al., 1994]. Several groups have recently reported the existence of novel genotypes in southeast Asian countries such as Thailand and Vietnam, and have shown these to be closely related to type 6a [Mellor et al., 1996; Tokita et al., 1994, 1995]. Whether or not these are to be classified as separate major genotypes or as further subtypes of HCV-6 has still to be decided [Doi et al., 1996; Mellor et al., 1996].

One potentially important reason for genotype identification is the observation that virus genotype, along with virus load and host-related factors (severity of liver disease), may influence the response to antiviral treatment [Hino et al., 1994; Dusheiko et al., 1994]. Consequently, a number of techniques have been developed to identify HCV genotypes in clinical specimens. The

Accepted for publication May 28, 1996.

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majority of these methods use the polymerase chain reaction (PCR), and involve three main procedures to distinguish between genotypes. These are 1) the use of type-specific primers [differential amplification, Okamoto et al., 1992, 1993], 2) the use of type-specific probes to hybridise with amplified HCV DNA after PCR [Stuyver et al., 1993, 1995; Tisminetzky et al., 1994], and 3) digestion of the amplified PCR product using combinations of restriction enzymes [Mellor et al., 1996; Davidson et al., 1995; McOmish et al., 1994]. Serologically based HCV typing methods are competitive or noncompetitive enzyme-linked immunosorbent assays (ELISAs) that detect antibody to type-specific epitopes in the core and NS4 regions [Bhattacherjee et al., 1995; Dixit et al., 1995; Zhang et al., 1995b; Simmonds et al., 1993c; Tanaka et al., 1994; Machida et al., 1992].

Previous studies have compared the accuracy of these different typing methods [Dixit et al., 1995; Lau et al., 1995], but these have not examined more recently discovered variants such as type 6 virus. In this study, we have carried out an extensive survey of samples from Hong Kong blood donors including a large proportion of type 6 infections, and a comparative evaluation of several genotyping assays.

MATERIALS AND METHODS

Samples

A total of 212 serum samples obtained from Hong Kong blood donors (Red Cross Blood Transfusion Service, Yaumatei, Hong Kong) were used in this study. All had previously been identified as either anti-HCV positive or indeterminate using the Abbott Matrix assay. Seventy-four of these samples were used for the comparison of different genotyping assays. Abbott Matrix results, donor sex, age, and serum alanine transaminase (ALT) levels were available for all 212 samples, and risk factors for infection could be identified in 43 cases.

RNA Extraction and Nested Reverse Transcription-PCR (RT-PCR)

Virus RNA was directly extracted from 100 μl serum using proteinase K/sarcosyl and phenol/chloroform. The RNA was precipitated in ethanol at −20°C overnight and dissolved in 25 μl DEPC-treated water prior to RT-PCR [Davidson et al., 1995].

5′ Noncoding region (5′NCR). RNA was reverse transcribed using the 5′ NCR-specific outer antisense primer 209, followed by nested PCR amplification involving outer primers 209 and 939 (sense) and inner primers 211 (antisense) and 940 (sense) [Chan et al., 1992].

Core. Core-specific antisense primer 410 was used for reverse transcription of extracted RNA. Outer primers used for PCR amplification were 410 and 954 (sense), followed by inner primers 953 (antisense) and 951 (sense) [Mellor et al., 1995].

NS4. Based on currently available HCV-6a NS4 sequences [Bhattacherjee et al., 1995], type-specific primers were designed for hemi-nested RT-PCR amplification. Antisense primer 867 was 5′TTCCACATRTGY TTNKSCCAGAA3′, with two sense primers 865 (5′CTGGAGGTTATCACNAGCACNTGG3′-outer) and 866 (5′RTCCTCGTGGGTGGAGTCCTNGC3′-inner)

RFLP Analysis

Amplified DNA was incubated with combinations of restriction endonuclease enzymes overnight, then electrophoresed through a 4% Metaphor agarose gel (containing 0.5 μg/ml ethidium bromide) in 1 × TBE. Restriction patterns of cleaved DNA were viewed on a UV transilluminator.

5′NCR RFLP. All PCR-positive samples were genotyped using a combination of restriction endonucleases *Hae*III/*Rsa*I and *Mva*I/*Hinf*I to distinguish between types 1-6 [McOmish et al., 1994]. As the 5′ NCR of type 6 group variants found in Southeast Asia has previously been shown to produce restriction patterns identical to those of type 1 isolates [Mellor et al., 1996], *amplified* DNA was also cleaved using enzymes *Dde*I/*Hpa*II, which are able to distinguish some, but not all, of these different variants from type 1. Subtyping of HCV types 1, 2, and 3 was carried out using enzymes *Bst*UI (type 1) and *Scr*FI (types 2 and 3) as previously described [Davidson et al., 1995].

Core RFLP. Samples identified as type 1 or 6 by RFLP in the 5′ NCR were amplified using primers from the core region and cleaved using the restriction endonucleases *Ava*I, *Sma*I, and *Hae*II [Mellor et al., 1996].

1-6 Serotyping Assay

The serotyping ELISA is a competitive assay using type-specific peptides from two antigenic regions of NS4 [Bhattacherjee et al., 1995].

Sequencing

Primary PCR products were reamplified with one of the primers biotinylated and then they were bound onto streptavidin-coated magnetic beads (Dynabeads M280, Dynal). DNA strands were denatured using 0.15 M NaOH and then separated magnetically. The bound strand was sequenced using the "Sequenase" kit version 2.0 (United States Biologicals, USB) according to the manufacturer's instructions.

Statistical and Phylogenetic Analyses

Statistical analysis was carried out using standard statistical software (SYSTAT). Phylogenetic trees were constructed using the PHYLIP program, NEIGHBOR [Felsenstein, 1993].

RESULTS

Comparison of Genotyping Methods

A total of 74 HCV-positive samples were genotyped using three different assays, each based on different regions of the genome. In addition, the majority of the samples were sequenced in the core region to confirm the genotype identification. The first genotyping assay was based on RFLP of amplified sequences from the 5′NCR, and three versions of this assay were used to analyse the samples. The original 5′NCR RFLP used enzymes *Hae*III/*Rsa*I and *Mva*I/*Hinf*I to identify geno-

TABLE I. Identification of Genotypes by RFLP and Sequencing in the 5′NCR, Core, and NS4 Regions

Final designation	No.	5′NCR[a] *HaeIII/RsaI*	*MvaI/HinfI*	*BstUI*	*ScrfI*	*DdeI/HpaII*	Core[a] *SmaI*	*AvaI*	Sequencing Core No.	Type	NS4 No.	Type
Type 1a	3	b	A	A	—	A	s1	a1	—	—	—	—
	1	b	A	A	—	A	s5	a1	1	1a	—	—
Type 1b	41	b	A	B	—	A	s1	a3	9	1b	—	—
	1	d	A	B	—	A	s1	a3	1	1b	—	—
	1	e	A	B	—	A	s1	a3	—	—	—	—
Type 2a	3	c	D	—	D	—	—	—	2	2a	—	—
Type 2b	2	d	D	—	F	—	—	—	—	—	—	—
Type 6a	21	h	B	—	—	D	s5	a12	20	6a	11	6a

[a]Restriction patterns classified according to previous analyses: 5′NCR [Davidson et al., 1995]; Core [Mellor et al., 1996].

types 1 through 6 [McOmish et al., 1994]. The other versions used included a modification of the assay involving the use of enzymes *DdeI/HpaII* to identify novel genotypes isolated from South East Asia, and a further assay used to subtype genotypes 1, 2, and 3 [Davidson et al., 1995]. The second genotyping assay was based on RFLP of the core region [Mellor et al., 1995] and was used to distinguish between type 1 and type 6 group variants that may otherwise have produced identical RFLP patterns in the 5′NCR. The third assay was a serotyping assay [Bhattacherjee et al., 1995] which used competitive binding to detect genotype-specific antibody to two antigenic regions in NS4.

The 74 samples were amplified by PCR using primers from the 5′NCR. Amplified DNA was cleaved using *HaeIII/RsaI* and *MvaI/HinfI*, allowing all but two of the samples to be provisionally genotyped as types 1, 2, and 6 (Table I). Two samples produced unusual combinations of RFLP patterns (A with *MvaI/HinfI* but d or e with *HaeIII/RsaI*) [Smith et al., 1995]. Subtyping was carried out on the type 1 samples using *BstUI* and indicated type 1a in 4 and type 1b in 43 of the 47 type 1 samples. Three type 2a and two type 2b samples were identified using *ScrFI*.

Restriction analysis with *DdeI/HpaII* revealed that none of the type 1 samples contained substitutions at positions −127 and −155 previously associated with some type 6 group variants [Mellor et al., 1996]. To confirm the genotype identifications of the 5′NCR RFLP assay, all of the type 1 and type 6 samples were amplified in the core region and cleaved with *SmaI* and *AvaI*. These enzymes distinguish type 1a (pattern a1 with *AvaI*) from type 1b (pattern a3) and other type 1 variants (patterns a2,a4) from type 6 and type 6 group variants (patterns a10-a13, sl/s5) [Mellor et al., 1996].

All of the samples identified as type 6 produced the pattern a12s5, confirming their identification. Amongst the four samples identified as type 1a by RFLP in the 5′NCR using *BstUI*, all produced the a1 pattern. Similarly, all of the type 1b samples identified using *BstUI* produced the a3 pattern. The two samples with unusual *HaeIII/RsaI* patterns (d, e) were typed as 1b using *AvaI* and *SmaI*, and this was confirmed for one of these samples (d) by sequencing in the core region. This analysis failed to reveal any samples of type 6 group variants apart from 6a, and indicates that genotyping in the 5′NCR is accurate in this particular population.

All but two of the samples were assayed for type-specific antibody to NS4 peptides [Bhattacherjee et al., 1995] (Table II). Of those samples with detectable antibody, all but one reacted specifically with the peptides of the genotype identified by RFLP. The only exception was a type 6 sample that reacted with type 1 peptides. This sample was confirmed as type 6 by sequencing in the core and NS4 regions. The reason for the inappropriate activity is unclear. Type-specific antibody was not detected in 18 samples, giving the serotyping assay an overall sensitivity of 75%.

To investigate whether the HCV-6a-specific peptides used in the serotyping assay fully represented the antigenic variability within the genotype, 11 type 6a samples were amplified and sequenced within this region. Results indicate that the type 6 region on which the NS4 peptides were based in the assay was highly conserved (Fig. 1). Variants of type 6a show highly restricted variability through the NS4 region, displaying only two to three nucleotide differences over the 275 bases sequenced. Relatively tight clustering of type 6a variants is observed upon phylogenetic analysis of the NS4 region, compared with the diversity amongst variants of other genotypes such as 1b and 4a (Fig. 2).

Genotype Distribution

HCV RNA was extracted from a further 138 serum samples from Hong Kong blood donors (total 212). Having established the accuracy of the 5′NCR genotyping assay in this population, we used this method (*HaeIII/RsaI* and *MvaI/HinfI* followed by *BstUI* (type 1) or *ScrFI* (types 2, 3) to identify the genotypes present in these samples (Table III). From the 212 positive sera, the two genotypes most frequently detected were types 1b (n = 127, 60%) and 6 (n = 57, 27%). Other genotypes identified were types 1a (n = 13), 2a (n = 8), 2b (n = 3), and 3a (n = 4). A mixed infection was observed in only one donor who was infected with both genotypes 3a and 6a.

Reactivity in the Abbott Matrix Assay

All ELISA positive samples were tested by the Abbott Matrix assay which uses antigens (core, NS3, and NS4)

TABLE II. Detection of Type-Specific Antibody in Samples From Hong Kong Blood Donors

Genotype	Serotype 1	2	6	NTS	NR	Total	Sensitivity (%)	Concordance (%)
Type 1a	3	0	0	0	1	4	75	100
Type 1b	33	0	0	6	4	43	77	100
Type 2a	0	2	0	1	0	3	67	100
Type 2b	0	2	0	0	0	2	100	100
Type 6a	1	0	13	5	1	20[a]	70	93
Total	37	4	13	12	6	72	75	98

[a]Two samples not serotyped due to lack of serum.

	1680–1692	1693–1708 (boxed)		1710–1727 (boxed)	1728–1752	SERO TYPE	O.D. IN WELL U	1	6
PT	GCVVIVGRVVLSG	KPAIIPDREVLYREFDEM	E	ECSQHLPYIEQGMMLAEQF	KQKALGLLQTASRQAEVIAPAVQTNW				
HK-4	.....C..IT.T.	...VV....I..QQ....	.	...K.I..LAE.QQI....	R..V.....ASAK...ELK...HSA.				
T3950	.....C...T.T.	...VV....I..QQ....	.	...R.I..LAE.QQI....	R..V.....ASAK...ELK...HSA.				
HK94	IT.T.	...VV..?.I..QQ....	.	...R.I..LAE.QQI....	R..VF....ASAK...EL	6	>2.0	1.14	>2.0
HK97	IT.T.	...VV..?.I..QQ..?.	.	...R.I..LAE.QQI....	R..V?....ASAK...EL	6	>2.0	0.25	0.64
HK99	IT.T.	...VV..?.I..QQ....	.	...RDI..LAE.QQI....	R..V.....ASAK...ELK...	6	>2.0	0.41	1.66
HK103	IT.?.	...VV..?.I..QQ....	.	...R.I..LAE.QQI....	R..V.....ASAK...ELK....S	6	>2.0	0.12	0.89
HK110	.C..IT.T.	...VV..?.I..QQ..?.	.	...R.I..LAE.QQI....	R..V...	6	>2.0	0.18	0.50
HK113	..E..C..IT.T.	...VV..?.I..QQ..?.	.	...R.I..LAE.QQI....	R..V.....ASAK...ELK...HSA.	6	>2.0	0.23	0.52
HK114	.C..IT.T.	...VV..?.I..QQ..?.	.	...R.I..LAE.QQI....	R..V.....ASAK...	1	>2.0	1.42	0.42
HK157	.C..IT.T.	...IV..?.I..QQ....	.	...R.I..LAE.QQI....	R..V.....ASAK...ELK.	6	>2.0	0.41	0.68
HK77	T.	...VV..?.I..QQ....	.	...R.I..LAE.QQI....	R..V?....ASAK...ELK.	6	1.93	0.09	0.97
HK150	.C..IT.T.	...VV..?.I..QQ....	.	...R.I..LAE.QQI....	R..V.....ASAK...ELK.R.I.	6	1.02	0.02	0.38
HK125	T.TS	...IV..?.I..QQ....	.	...R.I..LAE.QQI....	R..V.....ASAK...ELK ..HSA.	6	0.57	0.07	0.17
HK185	..IT.T.	...VV..?.I..QQ..K.	.	...R.I..LAE.QQI....	R..V.....ASAK...ELK..	Not done			

Fig. 1. Comparison of HCV-6a amino acid sequences in the NS4 region with the prototype sequence (HCV-PT; Choo et al., 1991) and corresponding serotyping results. Antigenic regions to which serotyping peptides are made are in boxes. Well U = "Unblocked", well 1 = type 1 and well 6 = type 6 specific antibody. (?), sequence not determined. ".": sequence identity to HCV-PT.

derived from type 1a sequences. All 11 sera from individuals infected with type 1a (Table IV) reacted with all antigens of the assay, but 11% of the type 1b sera and 32% of the type 6 sera were unreactive with the NS4 antigen. No significant differences in reactivity were found between genotypes in the core or NS3 proteins.

Epidemiological and Clinical Background of Donors

HCV-positive blood donors were predominantly male (male/female ratio of 3:1; Table III) and this ratio was even higher within the type 6 group (5:1). The sample groups for genotypes 1a, 2a, 2b, and 3a were too small to detect any significant trend in these cases. No significant differences were found in the ages of donors infected with different genotypes (Fig. 3) and the range of ages for infected donors (16 to 59 years) was similar for all genotypes.

Median ALT values for all genotypes fell within the normal range (7-53 U/L) with the exception of type 2b (which are based on very few values) (Fig. 4). Although the ALT values of patients infected with type 6 were slightly lower than those infected with type 1b, this was not found to be statistically significant using Spearmans rank correlation ($P = 0.094$).

Risk factors were obtained from 43 HCV-positive donors. These include intravenous drug abuse (prior to 1984), blood transfusion (prior to 1989), tattooing, and acupuncture. In many cases, donors were identified as having been exposed to more than one risk factor. However, in donors with only one identifiable risk factor, 66% of those infected with HCV-6 had a history of drug abuse, compared to 7% donors with type 1b, while 33% HCV-6 infected donors had been previously transfused compared to 83% of those with type 1b (Table III).

DISCUSSION

The identification of HCV genotypes is important in the investigation of the natural history and clinical outcome of HCV infection. Many different methods which have various advantages and disadvantages, exist to identify genotypes. Although assays should be sensitive and specific, other important considerations include cost, time consumption, and ease of use. In our study, all of the genotyping methods used involved the analysis of amplified HCV cDNA by RFLP, while serotyping was carried out by means of a competitive ELISA using synthetic peptides from two antigenic regions of NS4. Although type 6 group variants, described as types 7–9 and 11 [Tokita et al., 1994, 1995; Mellor et al., 1996], have also been detected in countries close to Hong Kong such as Vietnam, no evidence for these variants was found in the Hong Kong population.

When used on the population of Hong Kong blood

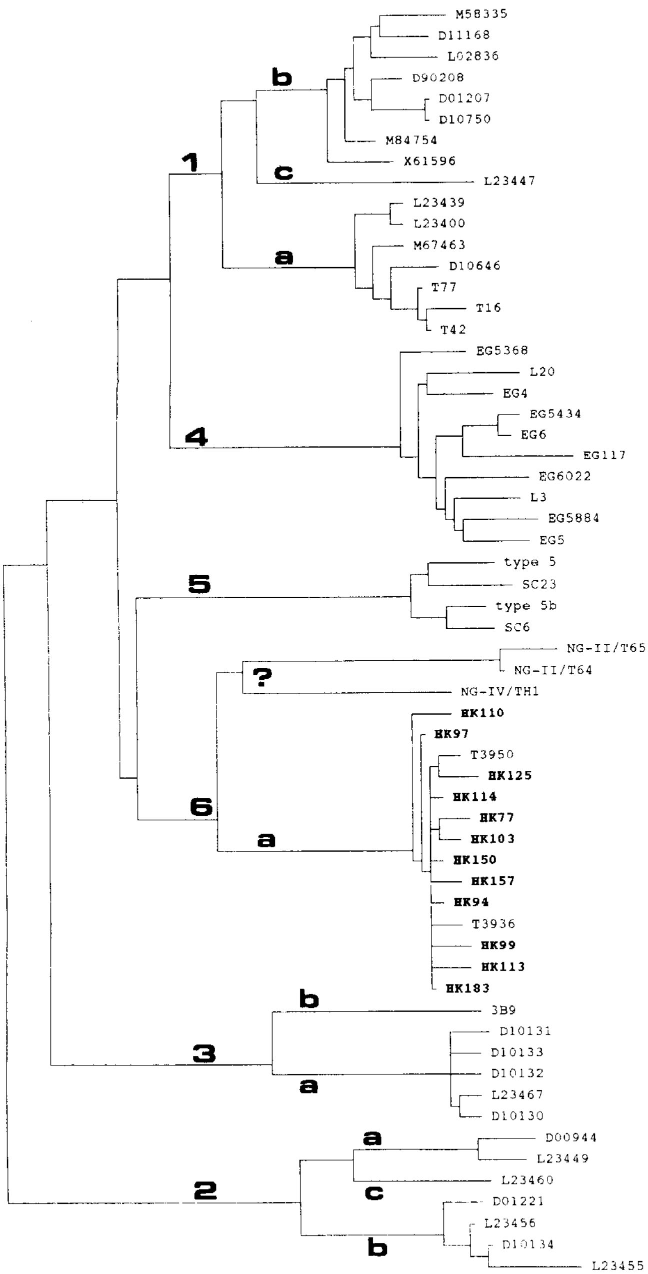

Fig. 2. Phylogenetic analysis of NS4 nucleotide sequences, showing HCV-6a samples from this study in bold.

donors, serotyping using NS4 peptides was found to be 75% sensitive. This low sensitivity may be explained by the observation that detection rates for types 1b and 6 are lower than for other genotypes [Bhattacherjee et al., 1995], at 83% (type 1b; compared to 96% for type 1a) and 67% (type 6). In our study, types 1b and 6 together accounted for 86% of the samples analysed. Higher sensitivities have been reported previously (87–90%) [Zhang et al., 1995b; Dixit et al., 1995; Simmonds et al., 1993c], although in these studies the range of virus genotypes was biased towards types 1a, 2a, 2b, and 3a.

The ELISA could be less sensitive to genotypes 1b and 6a if the peptides used did not fully represent the antigenic variability within those genotypes. However, sequence analysis indicated that this region is highly conserved within most type 6 viruses from Hong Kong (Fig. 1). Another possibility is that certain variants of these genotypes possess sequence changes which result in greater cross reactivity with other genotypes. For example, peptides in antigenic region 1 of type 6 are similar to those of types 1, 4, and 5 [Bhattacherjee et al., 1995]. Further sequence analysis of samples giving nontype-specific results in the NS4 region should reveal the cause of such reactivity.

Within Hong Kong, Macau, and Vietnam, type 6a was responsible for a significant proportion of HCV infections. In this study of HCV infected Hong Kong blood donors, 27% were found to be infected with type 6a. This figure was comparable with that reported from a previous, smaller survey in which HCV-6a accounted for 32% infections in blood donors [McOmish et al., 1994]. In contrast, an investigation of anti-HCV-positive patients in Hong Kong found only 14% to be infected with type 6a [Zhang et al., 1995a]. The fact that fewer patients are infected with type 6a than blood donors suggests that infections with this genotype may have been present in the population for a shorter time period than other genotypes, such as type 1b. Alternatively, it may be that type 6 is less pathogenic than type 1. Differences between genotypes 1 and 2 in their propensity to cause disease have been reported by some [Dusheiko et al., 1994; Booth et al., 1995], but not all investigations [Mahaney et al., 1994; Nousbaum et al., 1995].

Seventy-six percent of HCV-positive blood donors from Hong Kong were male, similar to the relative proportions of male and female subjects donating blood in this population. Similar frequencies of males and females were observed in type 1b infected donors (75%), while a slightly increased proportion of males were infected with type 6a (84%).

A history of drug abuse was reported in 66% of donors infected with HCV-6a, compared to only 7% of donors infected with type 1b. These results differ from a recent survey of HCV infected patients from Hong Kong [Zhang et al., 1995a]. Of eight patients infected with type 6, the source of infection was identified as blood transfusion/renal dialysis in seven and as occupational exposure in the remaining one.

The median ALT level of type 6 infected donors was lower than with type 1b (Fig. 4). Many studies have reported no significant difference in ALT levels associated with infections caused by different genotypes [Dhaliwal et al., 1996; Smith et al., 1996; Dusheiko et al., 1994], although other groups have identified distinct variations among ALT levels, particularly with type 3. However, these findings have been inconsistent, as HCV-3 has been associated with abnormal ALT more often than HCV-1 and HCV-2 [McOmish et al., 1993; Preston et al., 1995], although it also has been connected with having lower ALT values than other genotypes in other studies [Lau et al., 1995].

The future control of HCV infection lies partly with

TABLE III. HCV Genotype Distribution and Clinical Data of Hong Kong Blood Donors

Genotype	No. positive (%)	Donor sex		Mean age (range)	Risk factors				
		Male (%)	Female (%)		Transfusion	IVDA[a]	Tattoo	Acupuncture	None
Type 1	137 (65.0)	103 (76)	34 (24)	34 (16–59)	16	3	5	1	9
HCV-1a	13 (6.2)	10 (77)	3 (23)	35 (16–47)	1	1	0	0	0
HCV-1b	124 (58.8)	93 (75)	31 (25)	33 (17–59)	15	2	5	1	9
Type 2	11 (5.2)	5 (45)	6 (55)	34 (25–48)	0	0	2	0	0
HCV-2a	8 (3.8)	2 (25)	6 (75)	31 (25–48)	0	0	2	0	0
HCV-2b	3 (1.4)	3 (100)	0 (0)	37 (34–39)	0	0	0	0	0
HCV-3a	4 (1.9)	4 (100)	0 (0)	35 (24–42)	1	2	1	0	0
HCV-6a	57 (27.0)	48 (84)	10 (16)	34 (16–52)	3	6	4	0	0
Mixed	1 (0.5)	1 (100)	0 (0)	n/a	0	1	1	0	0

[a]Intravenous drug abuse.

TABLE IV. Reactivity of Blood Donor Samples in Abbott Matrix Assay According to Genotype

Genotype	Total no. samples	Number of reactive samples to Matrix antigens (%)				
		All antigens	Core	NS3	NS4 (yeast)	NS4 (*E. coli*)
1a	13	13 (100)	13 (100)	0 (0)	0 (0)	0 (0)
1b	125	110 (88)	124 (99)	0 (0)	111 (89)	116 (93)
2a	8	3 (38)	8 (100)	0 (0)	3 (38)	4 (50)
2b	3	2 (67)	3 (100)	0 (0)	2 (66)	2 (66)
3a	4	2 (50)	4 (100)	0 (0)	2 (50)	2 (50)
6a	57	38 (67)	57 (100)	1 (2)	39 (68)	42 (74)

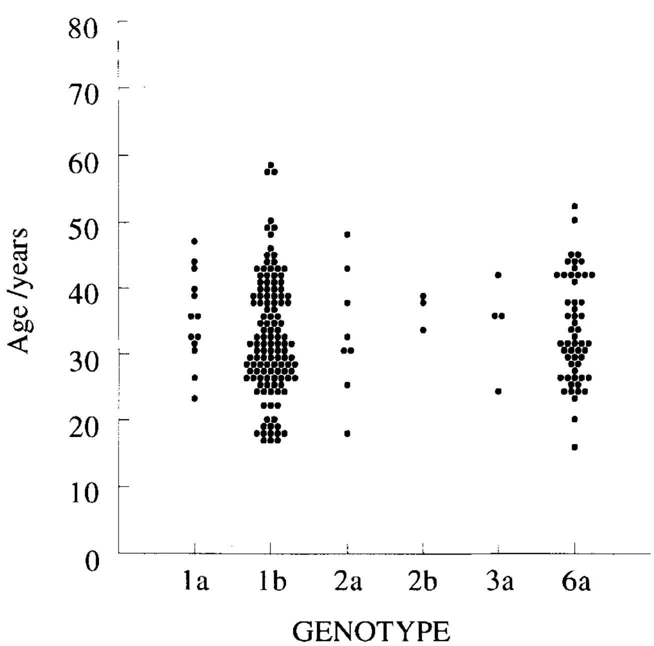

Fig. 3. Age distribution of blood donors infected with different genotypes in Hong Kong.

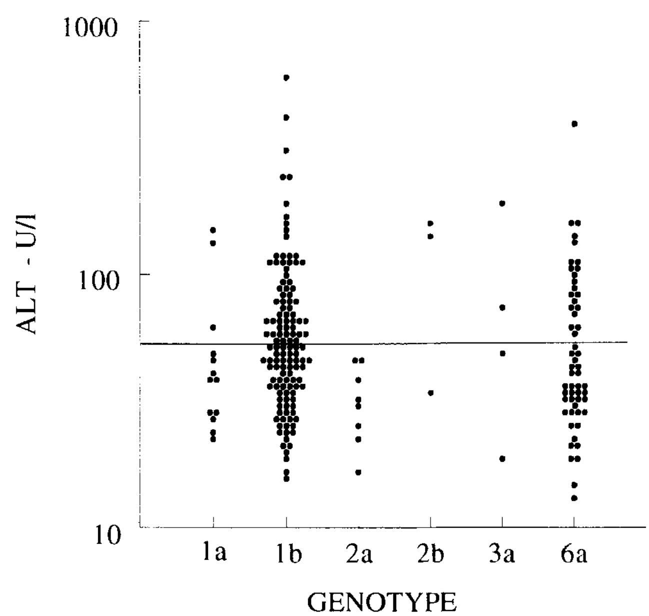

Fig. 4. Comparison of ALT values of donors infected with different genotypes in Hong Kong. Line indicates upper normal range (U/l).

the successful screening and detection of the virus among the blood donor population. Screening assays today use antigens based on sequences from a type 1a prototype virus, and a major concern is that these assays are less effective in the detection of nontype 1 infections [Dhaliwal et al., 1996]. The Abbott Matrix assay was used in the initial screening of these samples, and a lack of sensitivity to type 6 was noted, with 32% being nonreactive to the NS4 antigen. The lack of reactivity to RIBA antigens 5-1-1 and c100-3 by divergent genotypes has previously been reported in other studies [McOmish et al., 1994], and gives rise to concerns that in countries where genotypes other than 1, 2, and 3 exist, routine screening of blood donors could be less effective. One possible safeguard against this would be to include antigens specific to genotypes known to exist in that

area. For example, a screening assay including antigens specific to type 6 as well as type 1 could be used for routine screening in Hong Kong, Macau, and Vietnam.

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